

In the specification:

◊◊ Please replace the text on page 78, lines 1-30, with the following:

Construction of Shh Cys-1 mutants. The 584 bp NcoI-XhoI restriction fragment carrying the His-tagged wild type Shh N-terminal fragment from p6H-SHH was subcloned into the pUC-derived cloning vector pNN05 to construct the plasmid pEAG649. Cys-1 mutants of soluble human Shh were made by unique site elimination mutagenesis of the pEAG649 plasmid template using a Pharmacia kit following the manufacturer's recommended protocol. In designing the mutagenic primers, if a desired mutation did not produce a restriction site change, a silent mutation producing a restriction site change was introduced into an adjacent codon to facilitate identification of mutant clones following mutagenesis. To avoid aberrant codon usage, substituted codons were selected from those occurring at least once elsewhere in the human Shh cDNA sequence. The following mutagenic primers were used: (1) for C1F: 5' GGC GAT GAC GAT GAC AAA TTC GGA CCG GGC AGG GGG TTC 3' (SEQ ID NO: 5), which introduces an ApoI site to make pEAG837; (2) for C1I: 5' GGC GAT GAC GAT GAC AAA ATA GGA CCG GGC AGG GGG TTC 3' (SEQ ID NO: 6), which loses an RsrII site to make pEAG838; and (3) for C1M: 5' GGC GAT GAC GAT GAC AAA ATG GGC CCG GGC AGG GGG TTC GGG 3' (SEQ ID NO: 7), which loses both RsrII and AvaII sites to make pEAG839. Mutations were confirmed by DNA sequencing through a 180 bp NcoI-BglII restriction fragment carrying the mutant SHH proteins' N-termini in plasmids pEAG837-839. Expression vectors were constructed by subcloning each mutant plasmid's 180 bp NcoI-BglII fragment and the 404 bp BglII-XhoI fragment from pEAG649 into the phosphatase-treated 5.64 kb XhoI-NcoI pET11d vector backbone of p6H-SHH. Presence of the introduced restriction site change was reconfirmed in the expression vector for each Cys-1 mutant (C1F in pEAG840, C1I in pEAG841, and C1M in pEAG842). Expression vectors were transformed into competent E. coli BL21(DE3)pLysS (Stratagene) following the manufacturer's recommended protocol and selected on LB agar plates containing 100 µg/ml ampicillin and 30 µg/mL chloramphenicol. Individual colonies were selected and transformed bacteria were grown to an A₆₀₀ of 0.4-0.6 and induced for 3 h with 0.5 mM IPTG. Bacterial pellets were analyzed for expression of the mutant proteins by reducing SDS-PAGE and by Western blotting.

◊◊ Please replace the paragraph beginning on page 78, line 31, with the following:

A soluble human Shh mutant with multiple N-terminal hydrophobic substitutions (C1II) was made by unique site elimination mutagenesis using a Pharmacia kit following the manufacturer's recommended protocol. In designing the mutagenic primers, if a desired mutation did not produce a restriction site change, a silent mutation producing a restriction site change was introduced into an adjacent codon to facilitate identification of mutant clones following mutagenesis. To avoid aberrant codon usage, substituted codons were selected from those occurring at least once elsewhere in the human Shh cDNA sequence. The following mutagenic primer was used on the C1F template plasmid pEAG837 for C1II: 5' GCG GCG ATG ACG ATG ACA AAA TCA TCG GAC CGG GCA GGG GGT TCG GG 3' (SEQ ID NO: 8), which removes an ApoI site to make pEAG872. Mutations were confirmed by DNA sequencing through a 0.59 kb NcoI-XhoI restriction fragment carrying the mutant C1II Shh. An expression vector was constructed by subcloning the mutant plasmid's NcoI-XhoI fragment into the phosphatase-treated 5.64 kb XhoI-NcoI pET11d vector backbone of p6H-SHH. Presence of the introduced restriction site change was reconfirmed in the expression vector for the C1II mutant, pEAG875. The expression vector was transformed into competent E. coli BL21(DE3)pLysS (Stratagene) following the manufacturer's recommended protocol and selected on LB agar plates containing 100 µg/mL ampicillin and 30 µg/mL chloramphenicol. Individual colonies were selected and transformed bacteria were grown to an A_{600} of 0.4-0.6 and induced for 3 h with 0.5 mM IPTG. Bacterial pellets were analyzed as described above to confirm expression of mutant Shh protein.

◊◊ Please replace the text on page 81, lines 4-18, with the following:

Construction of the C1II/A169C mutant. The soluble human Shh mutant C1II/A169C (with cysteine substituted for the dispensable C-terminal residue A169 which is predicted to have a high fractional solvent accessibility) was made by unique site elimination mutagenesis using a Pharmacia kit following the manufacturer's recommended protocol and employing the mutagenic

oligo design principles described above. The following mutagenic primer 5' GAG TCA TCA GCC TCC CGA TTT TGC GCA CAC CGA GTT CTC TGC TTT CAC C 3' (SEQ ID NO: 9) was used on C1II Shh template pEAG872 to add an FspI site to make pSYS049. The C1II/A169C mutations were confirmed by DNA sequencing through a 0.59 kb NcoI-XhoI restriction fragment. The expression vector pSYS050 was constructed by subcloning the NcoI-XhoI fragment into the phosphatase-treated 5.64 kb XhoI-NcoI pET11d vector backbone of p6H-SHH. Presence of the introduced restriction site change was reconfirmed in the expression vector. The expression vector was transformed into competent E. coli BL21(DE3)pLysS, colonies were selected, induced, and screened for Shh expression as described above.